

The *MnSOD* Val/Val Genotype Enhances Lung Cancer Risk by *p53* and *XRCC1* polymorphisms

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Abstract

BACKGROUND: Exogenous ROS (reactive oxygen species) can induce DNA damage and cancer initiation. *MnSOD* (manganese superoxide dismutase) catalyzes the dismutation of a major type of ROS, i.e. superoxide radicals, into hydrogen peroxide. *p53* is a tumor suppressor protein, and *XRCC1* (X-ray cross-complementing group 1) is involved in the base-excision repair of ROS-induced DNA damage.

METHODS: To investigate whether the *MnSOD* Ala16Val polymorphism may modify the associations between *p53* Arg72Pro and *XRCC1* Arg399Gln polymorphisms and lung cancer risk, we carried out a case-control study with 935 Caucasian NSCLC (non-small cell lung carcinoma) patients and 1233 controls. The results were analyzed using logistic regression models, adjusting for possible confounding variables.

RESULTS: There was no association between the *p53* or *XRCC1* polymorphism and NSCLC risk for individuals with *MnSOD* Ala/Ala or Ala/Val genotype. For individuals with *MnSOD* Val/Val genotype, higher risks were found for *p53* (variant Pro allele vs. Arg/Arg), *XRCC1* (variant Gln allele vs. Arg/Arg), and the combination of two polymorphisms ("double-variant" vs. "double-wild-type"), with the adjusted odds ratios (ORs) of 1.84 (95% confidence interval, 1.2-2.8), 1.39 (95% CI, 0.9-2.1), and 2.54 (95% CI, 1.4-4.7), respectively. Furthermore, higher risk of the "double-variant" in *MnSOD* Val/Val genotype group was specific for adenocarcinoma cases only, and not for squamous cell carcinoma cases, with the adjusted ORs of 3.31 (95% CI, 1.7-6.5) and 0.69 (95% CI, 0.2-2.0), respectively.

CONCLUSIONS: The *MnSOD* Val/Val genotype may increase the NSCLC risk of *XRCC1* and *p53*, and combination of the two polymorphisms were associated with an even higher risk of NSCLC, specifically adenocarcinoma.

Introduction

Function of Protein/Enzyme

MnSOD, *p53*, and *XRCC1* play important roles in the defense of various damages induced by ROS (reactive oxygen species)

Tobacco smoke is one major source of ROS. Accumulation of ROS may stimulate cell proliferation and damage DNA, leading to the initiation or promotion of cancer

- MnSOD*, the only known superoxide scavenger in mitochondria, catalyzes the dismutation of a specific type of ROS, superoxide radicals, into hydrogen peroxide and oxygen

- p53* is a tumor suppressor protein involved in multiple pathways including apoptosis, cellular transcriptional control, and cell cycle regulation

- XRCC1*, one of more than 20 proteins that participate in the basal excision repair pathway, has multiple roles in repairing ROS mediated basal DNA damage and single strand DNA breaks

Function of Polymorphisms

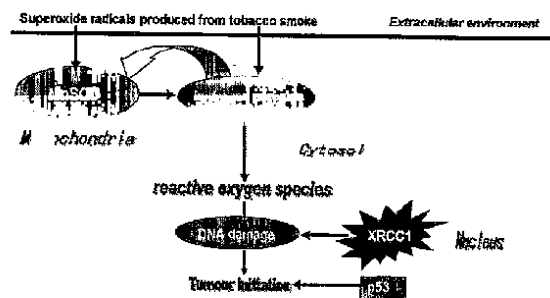
MnSOD, *p53*, and *XRCC1* are all polymorphic genes where each has been associated independently with the risk of lung cancer.

- The variant Val allele of the *MnSOD* Ala16Val polymorphism produces a conformational change in the helical structure of the protein. The variant genotype of this polymorphism may result in decreased efficiency of transport into mitochondria, and is associated with higher risk of lung cancer.
- The variant Pro allele of the *p53* Arg72Pro polymorphism has been associated with reduced apoptotic kinetics and higher risk of lung cancer, specifically adenocarcinoma.
- The Gln allele of the *XRCC1* Arg399Gln polymorphism is associated with higher levels of DNA, higher sister chromatid exchange frequencies, and higher risk of lung cancer. These associations with lung cancer risk have been shown to be modified by cigarette smoking habits.

Hypothesis:

- The *MnSOD* polymorphism may modify the associations either between *p53* Arg72Pro or *XRCC1* Arg399Gln polymorphism and the risk of non-small cell lung cancer. In other words, the *MnSOD* Val/Val genotype may enhance the lung cancer risk by *p53* and *XRCC1* polymorphisms.
- Various histological cell types of NSCLC may have different associations with the joint effects of the three polymorphisms

MnSOD, *p53*, *XRCC1*, and cigarette smoking



Materials and Methods

Design

Hospital Based Case-Control Study

Study Population

Cases:

- Histologically confirmed incident NSCLC (Non-Small Cell Lung Carcinoma) patients at Massachusetts General Hospital (1992-2000)

Controls:

- Friends or spouses of lung cancer patients or of other cardiothoracic surgery patients in the same hospital
- Where possible, friends were recruited in favor of spouses
- No particular matching characteristics between cases and controls
- The distribution of smoking variables in our controls was similar to the general Massachusetts population > age 45

Data Collection

1. Interviewer-administered questionnaires on demographic, and smoking histories.

2. Peripheral blood samples were obtained from each subject.

Genotyping for *MsSOD*, *p53* and *XPC/HR23C* polymorphism

1. Genotyping was performed blinded to case status.
2. PCR-sequencing method for *MsSOD* polymorphism (Wang et al., 2001)
3. PCR-RFLP method for *p53* and *XPC/HR23C* polymorphism (Jin et al., 2001; Duij et al., 2001)
4. A random 6% of the samples were repeated to assess the reproducibility of results.

Statistical Analysis

1. We analyzed all Caucasians with complete information on age, gender, smoking status (non-, ex- and current smokers), pack-years of smoking, and years since smoking cessation (for ex-smokers).
2. Generalized Additive models suggested that analyses should incorporate square root of pack-years and log-transformed cigarettes per day in place of their untransformed values, where appropriate.
3. In each analysis, the heterozygous and homozygous variant genotype groups of *p53* (Arg/Pro and Pro/Pro) and *XPC/HR23C* (Arg/Arg and Glu/Glu) were combined as *p53* variant and *XPC/HR23C* variant, respectively, because of the low frequency of the homozygous variants and the similar risk.
4. Stratified multiple logistic regression model by *MsSOD* genotypes were fitted to analyze associations between *p53*, *XPC/HR23C*, and combined *p53* and *XPC/HR23C* polymorphisms and NSCLC.
5. Adjusted models include covariates for age, genotypes, smoking status (non-, ex- and current smokers), SR-PY (square root of pack-years), and years since smoking cessation.
6. Subgroup analyses were performed in different age, pack-year, histological cell type, and clinical stage strata.

Table 1: Demographic Information by Case Status

Characteristics	Cases 935	Controls 1233	P
Age ¹	67 (30-61)	69 (18-100)	<0.01
Gender ²			
male	500 (53%)	587 (48%)	
female	435 (47%)	646 (52%)	<0.01
Smoking Status ³			
never	56 (6%)	432 (35%)	
ex-smoker	507 (54%)	562 (46%)	
current smoker	372 (40%)	239 (19%)	<0.01
Pack-years ^{1,3}	54 (0.1-231)	26 (0.1-210)	<0.01
Cigarettes/day ^{1,3}	30 (1-100)	20 (1-100)	<0.01
Smoking duration ^{1,3}	40 (0.5-73)	27 (0.1-65)	<0.01
Years since quitting smoking ^{1,4}	12 (1-59)	18 (1-56)	<0.01
<i>MsSOD</i> genotype ²			
Ala/Ala	208 (22%)	322 (26%)	
Ala/Val	472 (51%)	628 (51%)	
Val/Val	255 (27%)	283 (23%)	<0.01
<i>p53</i> genotype ²			
Arg/Arg	498 (53%)	718 (58%)	
Arg/Pro	367 (39%)	431 (35%)	
Pro/Pro	72 (8%)	83 (7%)	0.04
<i>XPC/HR23C</i> genotype ²			
Arg/Arg	400 (43%)	551 (45%)	
Arg/Glu	397 (42%)	539 (44%)	
Glu/Glu	138 (15%)	143 (11%)	0.04

¹ For age, pack-years and number of cigarettes per day, and years since smoking cessation, the data is reported as median (range), and based by the median test.

² Genotypes were determined by PCR-sequencing and PCR-RFLP methods.

³ Smokers: individuals who have never smoked.

Table 2: Genotype frequencies of *p53* and *XPC/HR23C* by *MsSOD* polymorphisms

	<i>MsSOD</i> Ala/Ala			<i>MsSOD</i> Ala/Val			<i>MsSOD</i> Val/Val		
	Case	Control	P	Case	Control	P	Case	Control	P
<i>p53</i>									
Arg/Arg	10 (33%)	16 (24%)	0.25	215 (57%)	243 (53%)	0.14	137 (60%)	153 (63%)	0.87
Arg/Pro	16 (47%)	124 (46%)		175 (38%)	203 (43%)		172 (78%)	200 (80%)	
Pro/Pro	13 (38%)	13 (5%)	0.01	23 (5%)	20 (5%)	0.74	22 (10%)	10 (4%)	0.10
<i>XPC/HR23C</i>									
Arg/Arg	45 (45%)	140 (34%)	0.01	210 (54%)	279 (40%)	0.001	93 (39%)	132 (52%)	0.001
Arg/Glu	50 (50%)	141 (34%)		224 (56%)	274 (39%)		117 (51%)	132 (52%)	
Glu/Glu	25 (25%)	40 (10%)	0.001	22 (5%)	22 (3%)	0.35	41 (18%)	43 (17%)	0.80
Combined <i>p53</i> and <i>XPC/HR23C</i>									
Wild-type + Wild-type	51 (51%)	75 (32%)	0.001	117 (30%)	155 (22%)	0.001	46 (20%)	70 (28%)	0.001
Wild-type + Mutant	59 (59%)	109 (48%)		153 (40%)	230 (33%)		76 (34%)	96 (39%)	
Mutant + Mutant	18 (18%)	38 (17%)	0.85	53 (14%)	128 (18%)	0.001	51 (23%)	54 (22%)	0.87
Wild-type + Wild-type	55 (55%)	75 (32%)	0.001	117 (30%)	155 (22%)	0.001	46 (20%)	70 (28%)	0.001

Cell counts are shown in parentheses.

¹ The *p* value indicates the significance of the difference between the Arg/Arg and Glu/Glu genotypes.

Table 3: Crude and adjusted odds ratios of the polymorphisms of *p53* and *XPC/HR23C* in different genotype groups of *MsSOD* polymorphism

	<i>MsSOD</i> Ala/Ala			<i>MsSOD</i> Ala/Val			<i>MsSOD</i> Val/Val		
	Crude	Adjusted	P	Crude	Adjusted	P	Crude	Adjusted	P
<i>p53</i>									
Arg/Arg	1.0	1.0		1.0	1.0		1.0	1.0	
Arg/Pro	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14
Pro/Pro	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14
Combined									
Wild-type + Wild-type	1.0	1.0		1.0	1.0		1.0	1.0	
Wild-type + Mutant	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14
Mutant + Mutant	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14	0.6 (0.4-1.2)	0.6 (0.4-1.2)	0.14

¹ Adjusted odds ratios are shown in parentheses.

² The odds ratios are shown in parentheses.

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Table 4. Stratified analyses of the combination of *p53* and *XRCC1* polymorphisms for individuals with the *MnSOD Val/Val* genotype^a

	Sample size	Odds ratio (95% CI) ^b	
	Case/Control	Crude	Adjusted
Age < 55	50/103	1.76 (0.7-4.5)	1.54 (0.5-4.7)
Age ≥ 55	205/182	2.55 (1.4-4.7)	3.59 (1.7-7.7)
Female	122/151	2.89 (1.4-5.9)	3.33 (1.4-7.9)
Male	133/134	1.95 (1.0-3.9)	1.60 (0.9-4.1)
Pack-years < 30	69/210	3.32 (1.4-7.8)	3.40 (1.3-8.7)
Pack-years ≥ 30	186/89	2.10 (0.9-4.7)	1.51 (0.8-4.5)
Adenocarcinoma cases vs. controls	150/285	3.21 (1.8-5.5)	3.31 (1.7-6.5)
Squamous cell carcinoma cases vs. controls	53/235	1.13 (0.5-1.8)	0.69 (0.2-2.0)
Early stage (I and II) cases vs. controls	153/235	2.15 (1.2-3.9)	2.45 (1.1-4.6)
Late stage (III and IV) cases vs. controls	101/225	2.90 (1.4-5.9)	2.78 (1.3-6.1)

^a Stratified analysis. In all of the analyses, the logistic regression model included the following variables: age, gender, square root of pack-years, smoking status, time since smoking cessation (in years), and genotype groups.

^b The odds ratio was "double-variant" (*p53* variant + *XRCC1* variant) vs. "double-wild-type" (*p53* Arg/Arg + *XRCC1* Arg/Arg) in individuals with the *MnSOD Val/Val* genotype.

Results

- No association was found between *p53* or *XRCC1* polymorphism and NSCLC risk for individuals with the *MnSOD Ala/Ala* or *Ala/Val* genotype.
- For individuals carrying the *MnSOD Val/Val* genotype, higher NSCLC risks were found for *p53* (variant Pro allele vs. Arg/Arg), *XRCC1* (variant Gln allele vs. Arg/Arg), and the combination of two polymorphisms ("double-variant" vs. "double-wild-type"), with the adjusted odds ratios (ORs) of 1.84 (95% confidence interval, 1.2-2.8), 1.39 (95% CI, 0.9-2.1), and 2.64 (95% CI, 1.4-4.7), respectively.
- Furthermore, higher NSCLC risk of the "double-variant" in the *MnSOD Val/Val* genotype group was specific for adenocarcinoma cases only, and not for squamous cell carcinoma cases, with the adjusted ORs of 3.31 (95% CI, 1.7-6.5) and 0.69 (95% CI, 0.2-2.0), respectively.

In the case-only analysis, the crude and adjusted ORs of adenocarcinoma vs. squamous cell carcinoma were 2.84 (1.15-6.99) and 2.58 (1.00-6.70), respectively.

Conclusions

- The *MnSOD Val/Val* genotype increases the NSCLC risk of *XRCC1* and *p53*, individually.
- The *MnSOD Val/Val* genotype increases the NSCLC risk of the combination of *XRCC1* and *p53* genotypes even further.
- Specifically, the increased risks were primarily seen in lung adenocarcinomas.

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